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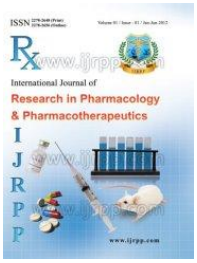

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Free radical scavenging and protease inhibitory activities of *Datura stramonium L*

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ABSTRACT

It is generally accepted that proteolytic enzymes are involved in the catabolic aspect of normal tissue remodeling and that altered activity of these enzymes is responsible for cartilage destruction and bone erosion associated with degenerative disorders such as rheumatoid arthritis (RA). In addition, reactive oxygen free radical species (ROS) have also been implicated in the pathogenesis of degenerative joint diseases. Hence the development of novel therapeutic strategies against rheumatoid arthritis should include both protease inhibitory and free radical scavenging elements. In the present study an in vitro evaluation of the free radical scavenging and protease inhibitory activities of the medicinal plant *Datura stramonium* L. has been attempted. Free radical scavenging activity was evaluated using the 1, 1-diphenyl, 2-picrylhydrazyl free radical (DPPH), 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and hydroxyl radical scavenging assays. The protease inhibitory activity was evaluated using the serine protease trypsin and the substrate BAEE (N-benzoyl-L-arginine ethyl ester).The plant extract showed significant radical scavenging activity with a percentage of inhibition of 51.8%, 78.7% and 67.7% for DPPH, ABTS and hydroxyl radical scavenging assays respectively. The plant also exhibited an inhibitory activity against protease with a percentage of inhibition of 58.30%. Results suggest that the plant extract possesses active principles capable of scavenging free radicals and inhibiting proteases that could be of therapeutic value in the management of rheumatoid arthritis.

KEYWORDS: *Datura stramonium* L., Rheumatoid arthritis, Free radical, protease.

INTRODUCTION

Free radicals are formed in both physiological and pathological conditions in mammalian tissues. The uncontrolled production of free radicals is considered as an important factor in the tissue damage induced by several pathophysiologies [1]. Oxidative stress has been described as an important mechanism that underlies destructive proliferative synovitis in rheumatoid arthritis [2-4]. Oxygen and nitrogen radicals damage cellular elements in cartilage and components of the extracellular matrix either by direct attack or indirectly by reducing matrix components synthesis (proteoglycans, Type II collagen), reducing the sulfation of newly synthesized glycosamino glycans, inducing apoptosis or by activation of latent metalloproteinases [5-6]. The scavenging of these free radicals by antioxidants plays a significant role in the therapeutic management of such conditions. Proteolytic enzymes liberated from polymorphonuclear leucocytes and other necrotizing tissues are responsible for much of the tissue damage in effected joints in inflammatory joint diseases [7-9]. Neutrophil serine proteinases

have been shown to break down both proteoglycan and collagen in articular cartilage [10-13]. Inhibition of these proteases by other normally occurring inhibitors as well as synthetic ones could be an important step in preventing tissue damage in rheumatoid arthritis. *Datura stramonium* L. is a widespread annual plant belonging to Solanaceae family. It is a popular folklore medicinal herb. It is a wild growing flowering plant and was investigated as a local source for tropane alkaloids which contain a methylated nitrogen atom (N-CH3) and include the anti-cholinergic drugs atropine, and scopolamine [14]. The leaves of *D. stromonium* L. are used for relief of headache and a vapour of leaf infusion is used to relive pain from rheumatism and gout. Smoke from burning the leaves is inhaled for relief of asthma and bronchitis. Various pharmacological activities including analgesic, anti-inflammatory, antidiarrhoeal, antiasthmatic, anticholinergic, antimicrobial, and larvicidal activities have been reported from the plant [15-17]. In the present study the methanolic extract of the leaves of *Datura stramonium* L. was evaluated for its radical scavenging property using the DPPH, ABTS and hydroxyl radical scavenging assays and for its protease inhibitory activity.

MATERIALS AND METHODS

PLANT MATERIAL AND EXTRACTION

Leaves of *Datura stramoniun* L. were collected from local areas of Kannur District, Kerala and identified at the Department of Botany, Govt. Brennan College, Kannur. The plant material were washed thoroughly, dried under shade and coarsely powdered. Preparation of extract was carried out by cold extraction method. The powdered plant material was extracted with methanol under constant stirring for 48 hours and filtered. The filtrate was collected and concentrated using rotary vacuum evaporator to obtain the crude extract which was used for further studies.

CHEMICALS

All solvents and chemicals used were of analytical grade. 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis-(3- ethyl benzothiazoline- 6 sulphonic acid) (ABTS) were purchased from Sigma , U.S.A. Trypsin, N-benzoyl-L-arginine ethyl ester (BAEE), Phenyl methylulphonyl Fluoride (PMSF) and ascorbic acid were purchased from Sisco Research Labortories, India.

FREE RADICAL SCAVENGING ASSAYS

DPPH RADICAL SCAVENGING ACTIVITY

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical and is very popular for the study of natural antioxidants. On accepting hydrogen from a corresponding donor, the solution loses the characteristic deep purple colour and discoloration was proportional to the concentration and scavenging activity of the compound. The reaction mixture contained 2.8 ml of 100µM DPPH dissolved in methanol and various concentrations (0.2 -1 mg/ml) of plant extracts in 0.2 ml methanol. After incubation at room temperature for 30 minutes the mixture was shaken and absorbance was measured at 517 nm. An equal amount of methanol and DPPH without sample served as control. Ascorbic acid was used as the standard. The percentage of DPPH scavenging activity was calculated as follows.

$$\%\ \text{of DPPH scavenging activity} = \frac{\text{A-B}}{\text{A}} \times 100$$

Where A is the absorbance of control (DPPH solution without the sample) and B is the absorbance of DPPH solution in presence of the sample (extract/ ascorbic acid).

ABTS RADICAL SCAVENGING ASSAY

ABTS assay is based on the ability of antioxidants to scavenge the long life radical cation $ABTS^{\cdot+}$ .This scavenging produces a decrease in the absorbance at 734nm [18]. The stock solutions included 7.4mM $ABTS^{\cdot+}$ solution and 2.6mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1mL $ABTS^{\cdot+}$ solution with 60mL methanol to obtain an absorbance of 1.1±0.02 units at 734 nm using the spectrophotometer. Fresh $ABTS^{\cdot+}$ solution was prepared for each assay. Plant extracts (150 µL) were allowed to react with 2850 µL of the $ABTS^{\cdot+}$ solution for 2 h under dark conditions. The absorbance was taken at 734nm using the spectrophotometer [19]. Ascorbic acid was used as positive control. The percentage inhibition was calculated according to the formula,

$$\%\ \text{of ABTS scavenging activity} = \frac{\text{A0-A1}}{\text{A0}} \times 100$$

Where A0 is the absorbance of the control and A1 is the absorbance of the sample.

HYDROXYL RADICAL SCAVENGING ASSAY

The hydroxyl radical scavenging assay is based on the ability of compounds to compete with salicylic acid for OH radicals. The reaction mixture contains 1 ml of 1.5 mM FeSO4, 0.7 ml of 6mM hydrogen peroxide, 0.3 ml of 20 mM sodium salicylate and 1 ml of various concentrations (0.2 - 1mg/ml) of the extracts. The mixture was incubated for 1 hour at 37°C and the absorbance of the hydroxylated salicylate complex was measured at 562 nm. Ascorbic acid was used as standard. The scavenging activity of hydroxyl radical is calculated as follows

% of Hydroxyl radical scavenging activity =1- (A1-A2 /A0)×100

Where A0 is absorbance of the control (without extract), A1 is the absorbance in the presence of the extract and A2 is the absorbance without sodium salicylate [20]

PROTEASE INHIBITORY ACTIVITY

Serine protease, trypsin was used to evaluate the protease inhibitory activity of the plant extracts. The assay is based on the hydrolysis of the substrate BAEE (N-benzoyl-L-arginine ethyl ester) at the ester linkage causing an increase of absorbance measured at 253nm.

BAEE + H_2O + Trypsin ⟶ N-α-Benzoyl-L-Arginine + Ethanol

The reaction mixture (3.4ml final volume) contained 67mM phosphate buffer (pH 7.6), 0.25mM BAEE in phosphate buffer and 0.05mM Trypsin in ice cold .001MHCl. Crude methanolic extract of *Datura stramoniun* was prepared in DMSO and used for the assay. The assay mixture containing 200µl of Trypsin and 200µl of test solution was incubated for 10 minutes. The reaction was initiated by the addition of 3ml of substrate and the absorbance was measured at 253nm for 10 minutes using UV visible spectrophotometer. PMSF (Phenyl methylulphonyl Fluoride) a known trypsin inhibitor was used as positive control. Graph showing the time-dependent activity of the enzyme was plotted. Trypsin inhibitory activity was expressed as percentage-inhibition of Trypsin, calculated by following the equation:

$$\% \text{ inhibition} = 1-\frac{B}{A} \times 100$$

Where A is the change in absorbance without test sample and B is the change in absorbance with the test solution.

RESULTS AND DISCUSSION

DPPH RADICAL SCAVENGING ASSAY

The DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to be decolorized in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. Scavenging activity of free radical DPPH has been widely used to evaluate the antioxidant activity of natural products from plant and microbial sources [21-22]. Fig. 1 shows the DPPH scavenging activity of the plant at different concentrations. The extract of *Datura stramonium* was able to reduce the stable radical DPPH to the yellow colored biphenyl picrylhydrazine and exhibited a notable dose dependent inhibition of the DPPH activity. The percentage of inhibition increased from 10.82% at a concentration of 0.2mg/ml to 51.8% at 1mg/ml thus indicating that the plant possesses good radical scavenging activity. The IC_{50} value was found to be 1.052mg/ml.

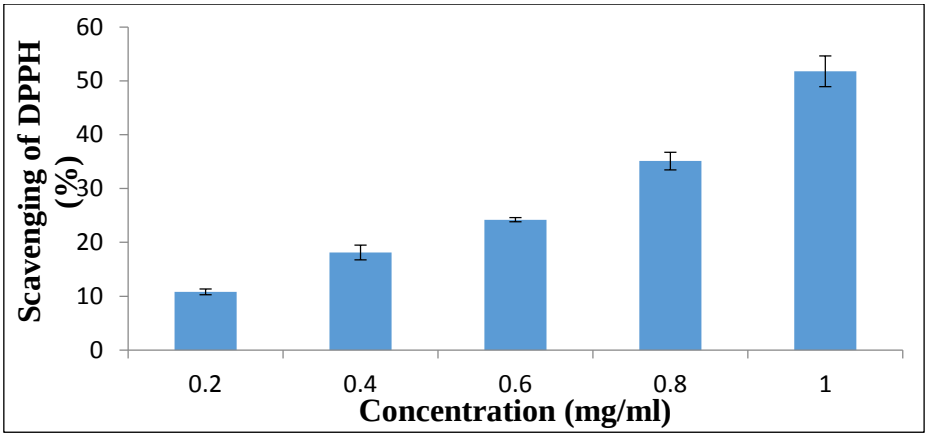

Fig.1: DPPH radical scavenging activity of methanolic extract of *Datura stramonium*

ABTS RADICAL SCAVENGING ASSAY

ABTS assay measures the relative ability of antioxidant to scavenge the ABTS generated in aqueous phase compared with a standard. The ABTS is generated by the reaction of a strong oxidising agent (eg. potassium permanganate or potassium persulphate) with the ABTS salt. Reduction of blue green ABTS radical solution by hydrogen donating antioxidant is measured by the suppression of its characteristic long wave (734nm) absorption spectrum. Evaluation of radical scavenging activity of plant sources by employing ABTS assay has been widely reported since it is applicable for both lipophilic and hydrophilic antioxidants [18]. In the present study the scavenging activity of the extract increased in a dose dependent manner (Fig.2). The scavenging of ABTS radical was found to increase from 24.14% to 78.7% at concentrations ranging from 0.2–1 mg/ml. The IC_{50} of the plant extract was found to be 0.550mg/ml. Therefore, the ABTS radical scavenging activity of methanol extract of *Datura stramonium* indicates its ability to scavenge free radicals, thereby preventing lipid oxidation via a chain-breaking reaction.

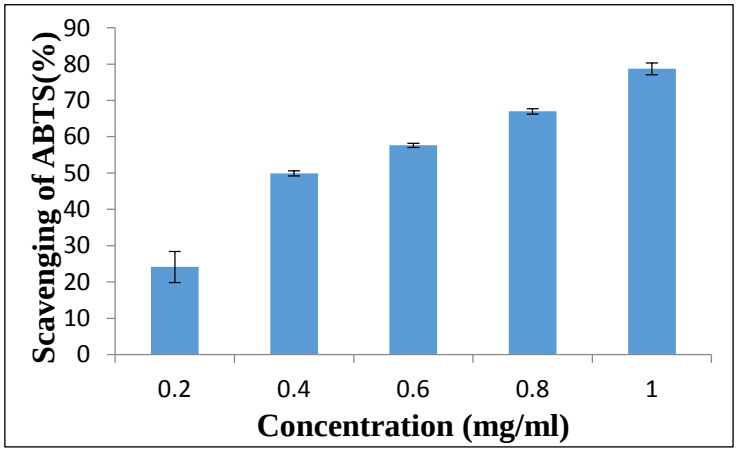

Fig. 2: ABTS radical scavenging activity of methanolic extract of *Datura stramonium*

HYDROXYL RADICAL SCAVENGING ACTIVITY

The hydroxyl radical is the most reactive of the reactive oxygen species, and it induces severe damage in adjacent biomolecules. The hydroxyl radical can cause oxidative damage to DNA, lipids and proteins. The importances of scavenging of hydroxyl radical in the management of various inflammatory conditions have been previously reported [23]. The •OH scavenging` activity of methanolic extract of *Datura stramonium* was assessed by its ability to compete with salicylic acid for •OH radicals in the •OH generating/detecting system. In the present study, the methanolic extract of *Datura stramonium* was found to scavenge hydroxyl radical significantly in a dose dependent manner. The hydroxyl radical scavenging effect of the plant extract at a concentration of 0.2mg/ml was found to be 39.29% and at a concentration of 1mg/ml was found to be

67.77%. The IC_{50} of the plant extract was found to be 0.6067mg/ml. Hence the plant extract can be considered as a good scavenger of hydroxyl radical.

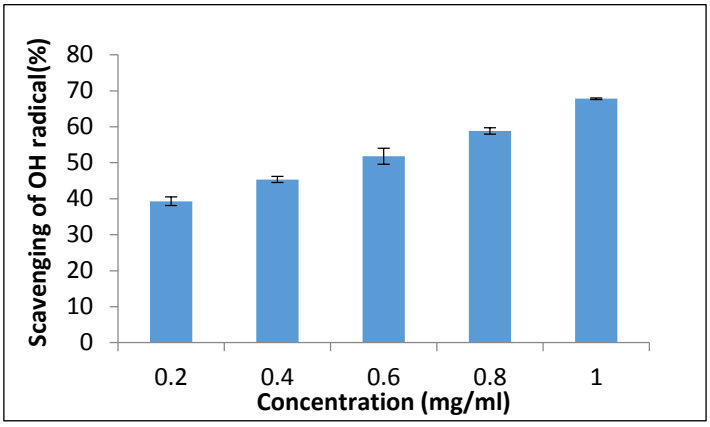

Fig. 3 : Hydroxyl radical scavenging activity of methanolic extract of *Datura stramonium*

PROTEASE INHIBITORY ACTIVITY

Proteases constitute one of the largest classes of potential drug targets. Protease inhibitors play a vital role in the regulation of protease activity. A number of protease inhibitors have displayed promising therapeutic activities in humans against viral and parasitic infections, cancer, inflammatory, immunological, respiratory, cardiovascular and degenerative disorders. It seems that the presence of protease inhibitors at the site of inflammation is important in limiting the destructive activity of proteases that are liberated from polymorphonuclear leucocytes and other necrotizing tissues. Previous studies on the isolation of protease inhibitors from plants such as Faba bean, arrow root, *Cassia fistula*, *Cicer arietinum*, *Moringa oleifera* etc. exhibiting wide pharmacological activities have been reported [24-28]. In the present study the protease inhibitory activity was performed using the crude methanolic extracts of *Datura stramonium*. Graph was plotted based on the values obtained at different time period (fig. 4). Percentage of Inhibition calculated by the formula was found to be 58.30%. The standard inhibitor PMSF showed an inhibition of 51.92%. The study indicates that the plant contain active constituents exhibiting potent antiprotease activity that could be of therapeutic value in the management of inflammatory joint diseases.

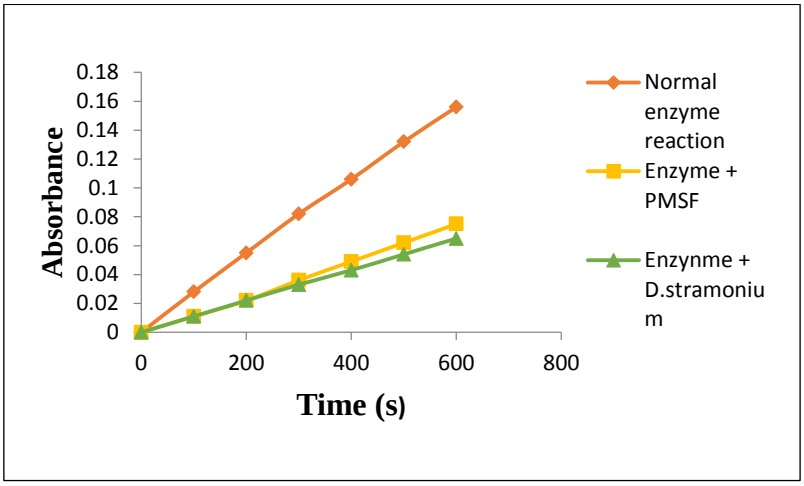

Fig. 4: Protease inhibitory activity of methanolic extract of Datura *stramonium*

CONCLUSIONS

In the present study, the free radical scavenging and protease inhibitory activity of the leaf extract of *Datura stramonium* L. was investigated. The plant extract exhibited good radical scavenging activity as evident from the results obtained from

DPPH, ABTS and hydroxyl radical scavenging assays. The plant extract also exhibited significant inhibition towards protease. Both these activities are of great significance since free radicals and proteolytic enzymes are likely to be of equal potential importance as damaging agents in the pathogenesis of inflammatory joint diseases including rheumatoid arthritis. Hence the design of novel therapeutic strategies for patients with such disorder should include both protease inhibitory and free radical scavenging elements. From the results obtained in the present study it can be concluded that the leaf extract of *Datura stramonium* L.possesses active principles capable of scavenging free radicals and inhibiting proteases that could be of therapeutic value in the management of rheumatoid arthritis. Further studies are required to isolate and identify the active constituents eliciting such pharmacological response.

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REFERENCES

[1]. Krishna Mohan Surapneni, V S Chandrasada Gopan. Lipid peroxidation and antioxidant status in patients with rheumatoid arthritis. Indian Journal of Clinical Biochemistry 2008, 23(1), 41-44.
[2]. Abbas Mirshafiey, Monireh Mohsenzadegan. The Role of Reactive Oxygen Species in Immunopathogenesis of Rheumatoid Arthritis. Iran J Allergy Asthma Immunol 2008, 7(4), 195-202.
[3]. Annil Mahajan, Vishal R Tandon. Antioxidants and rheumatoid arthritis. J Indian Rheumatol Assoc 2004, 12, 139-142.
[4]. F. Karatas, I. Ozates, H. Canatan, I. Halifeoglu, M. Karatepe, R. Colak. Antioxidant status & lipid peroxidation in patients with rheumatoid arthritis. Indian J Med Res 2003, 118, 178-181.
[5]. Carol A Hitchon, Hani S El-Gabalawy. Oxidation in rheumatoid arthritis. Arthritis Res Ther 2004, 6, 265-278.
[6]. K Bauerova, S Bezek. Role of Reactive Oxygen and Nitrogen Species in Etiopathogenesis of Rheumatoid Arthritis. Gen Physiol Biophys 1999, 18, 15-20.
[7]. D. Brackertz, J. Hagmann, F. Kueppers. Proteinase inhibitors in rheumatoid Arthritis. Ann. rheum. Dis 1975, 34, 225.
[8]. David Mantle, Gavin Falkous, David Walker. Quantification of protease activities in synovial fluid from rheumatoid and osteoarthritis cases: comparison with antioxidant and free radical damage markers. Clinica Chimica Acta 1999, 284, 45–58.
[9]. Leena Peltonen, Jaakko Puranen, Kari Lehtinen, L. Kalevi Korhonen. Proteolytic enzymes in joint destruction. Scand J Rheumatology 1981, 10, 107-114.
[10]. W. Winn Chatham, Richard Swaim, Henry Frohsin, Louis W. Heck, Edward J. Miller, Warren D. Blackburn. Degradation of human articular cartilage by neutrophils in synovial fluid. Arthritis and Rheumatism 1983, 36, 51-58.
[11]. I. C. Kowanko, E. J. Bates, A. Ferrante. Mechanisms of human neutrophil-mediated cartilage damage in vitro: The role of lysosomal enzymes, hydrogen peroxide and hypochlorous acid. Immunol Cell Biol 1989, 67, 321-329.
[12]. Phyllis M. Starkey, Alan J. Barrett, Mary C. Burleigh. The degradation of articular collagen by neutrophil proteinases. Biochimica et Biophysica Acta 1977, 483, 386-397.
[13]. Alan J. Barrett. The Possible Role of Neutrophil Proteinases in Damage to Articular Cartilage. Agents and Actions 1978, 8, 1-2.
[14]. Das Sanjita, Puneet kumar, Basu S.P. Phytoconstituents and therapeutic potentials of *Datura stramonium* Linn. Journal of Drug Delivery & Therapeutics2012, 2(3), 4-7.
[15]. Priyanka Soni, Anees Ahmad Siddiqui, Jaya Dwivedi,Vishal Soni. Pharmacological properties of *Datura stramonium* L. as a potential medicinal tree: An overview. Asian Pac J Trop Biomed 2012, 2(12), 1002-1008.

[16]. Maibam Rasila Devi, Meenakshi Bawari, S.B Paul, G.D.Sharma. Neurotoxic and Medicinal Properties of *Datura stramonium* L. – Review. Assam University Journal of Science & Technology 2011, 7(I), 139-144.
[17]. Duraid A Abbas. Analgesiac, anti-inflammatory and antidiarrhoeal effects of *Datura stramonium* hydroalcoholic leaves extract in mice. International Journal of Research and Reviews in Applied Sciences 2013, 14(1), 193-199.
[18]. Maryam Nasiri Aghdam, Gholamreza Dehghan, Hanieh Rahbar Kafshboran. Comparative study of ABTS radical scavenging activity and flavonoid contents in several populations of *Teucrium polium*. International proceedings of chemical, biological and environmental engineering 2011, 3, 55-58.
[19]. Kriengsak Thaipong, Unaroj Boonprakob, Kevin Crosby, Luis Cisneros-Zevallos, David Hawkins Byrne. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 2006, 19, 669–675.
[20]. G.sudha, M.Sangeetha Priya, R.Indhu Shree, S.Vadivukkarasi. In vitro free radical scavenging activity of raw pepino fruit (*Solanum muricatum* Aiton). Int J Curr Pharm Res 2011, 3(2), 137-140.
[21]. Vinay R. Patel, Prakash R. Patel, Sushil S. KajalAntioxidant Activity of Some Selected Medicinal Plants in Western Region of India. Advances in Biological Research 2010, 4 (1), 23-26.
[22]. Nooman A. Khalaf, Ashok K. Shakya, Atif Al-othman, Zaha El-agbar, Husni Farah. Antioxidant Activity of Some Common Plants. Turk J Biol 2008, 32, 51-55.
[23]. Comparative evaluation of Metal Chelating, Antioxidant and Free Radical Scavenging activity of TROIS and six products commonly used to control pain and inflammation associated with Arthritis. International Journal of Drug Development & Research 2011, 3(4), 208-216.
[24]. Evandro Fei Fang, Abdallah Abd Elazeem Hassanien, Jack Ho Wong, Clara Shui Fern Bah, Saeed Saad Soliman, Tzi Bun Ng. Isolation of a New Trypsin Inhibitor from the Faba Bean (*Vicia faba* cv. Giza 843) with Potential Medicinal Applications. Protein & Peptide Letters 2011, 18, 64-72.
[25]. N. Mallikarjuna Rao, H. Nayana Rao ,T. N. Pattabiraman. Enzyme inhibitors from plants. Isolation and characterization of a protease inhibitor from arrow root (*Maranta arundinaceae*) tuber. J. Biosci 1983, 5(1), 21-33.
[26]. I. Arulpandi, R. Sangeetha. Antibacterial Activity of Fistulin: A Protease Inhibitor
[27]. Purified from the Leaves of *Cassia fistula*. International Scholarly Research Network Pharmaceutics 2012, 1-4.
[28]. Meera Nair, Sardul Singh Sandhu, Anita Babbar. Purification of trypsin inhibitor from seeds of *Cicer arietinum* (L.) and its insecticidal potential against *Helicoverpa armigera* (Hübner). Theoretical and Experimental Plant Physiology 2013, 25(2), 137-148.
[29]. B. Bijina, Sreeja Chellappan, Jissa G. Krishna, Soorej M. Basheer, K.K. Elyas, Ali H. Bahkali, M. Chandrasekaran. Protease inhibitor from *Moringa oleifera* with potential for use as therapeutic drug and as seafood preservative. Saudi Journal of Biological Sciences 2011, 18, 273–281.